

## REMARKS

Rejection of Claims and Traversal Thereof

In the January 13, 2009 Office Action:

1. Claims 1, 4-10, 12-16 and 18-27 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lockhart et al., (WO 97/27317), in view of Lakowicz et al., (Photon Spectra (October 2001) 35(10): 96-104 (Note: the lead author is Lakowicz and not Gryczynski, et al.); and in further view of Cao, et al., (Journal of the Amer. Chem. Soc. (July 2001) 123: 7961-7962 and in further view of Qi et al., (Applied and Environmental Microbiology (2001) 67(8): 3720-3727; and
2. Claims 1, 4-10, 12-16 and 18-27 were rejected under 35 U.S.C. 103(a) as being unpatentable over Cao, et al., (Nanoparticles within Raman Spectroscopic Fingerprints for DNA and RNA Detection, Science, Aug 2002, Vol. 297, pp 1536-1540, hereinafter Cao); as evidenced by Malicka, et al., (Biopolymers (2003) 72(2) 96-104, hereinafter Malicka) and Lukomska et al., (Biopolymers and Biophysical Research Communication (2005) 328: 78-84) in view of Lakowicz 1 (US Patent Application No. 2002/0160400), and in further view of Lakowicz 2, (Radiative Decay Engineering: Biophysical and Biomedical Applications," Analytical Biochemistry, 2001, Vol. 298, pp 1-24, hereinafter Lakowicz 2).

These rejections are hereby traversed and reconsideration of patentability of the pending claims is therefore requested in light of the following remarks.

Rejections under 35 U.S.C. 103 (a)

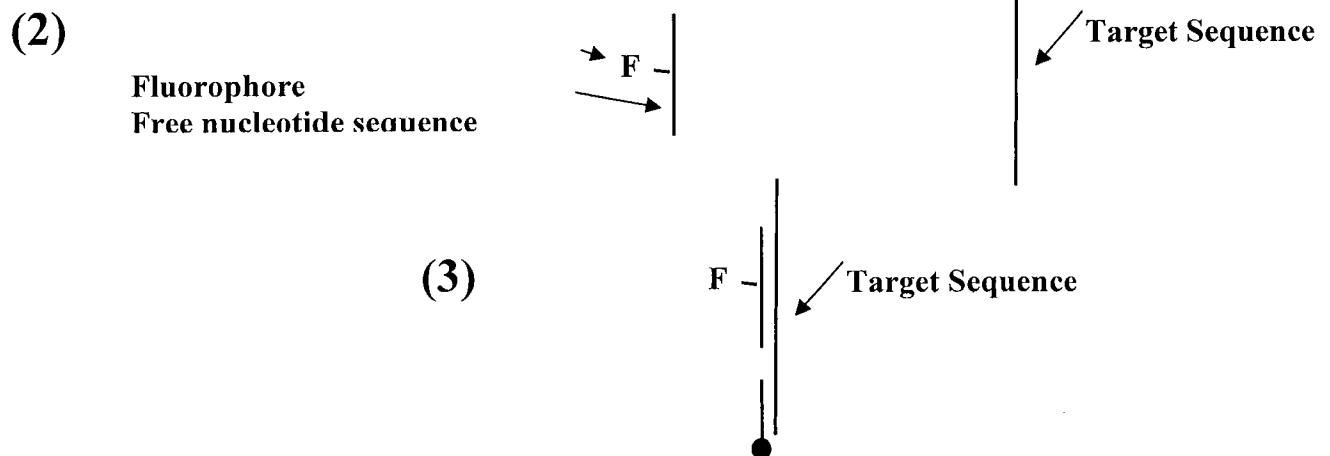
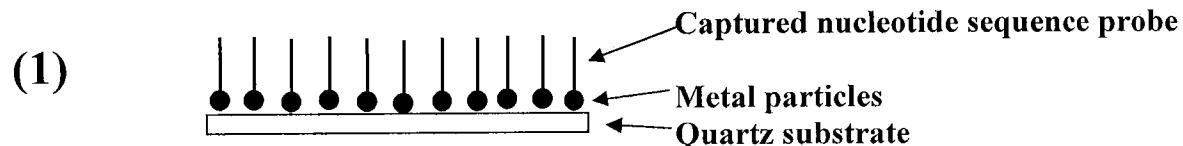
Claims 1, 4-10, 12-16 and 18-27 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lockhart et al., in view of Lakowicz et al., Cao, et al., and Qi et al. Applicants submit that the proposed combination does not in any way, disclose, teach or suggest the presently claimed invention.

The Office mistakenly believes that Lockhart describes all the limitations of claim 1 excepting the positioning of the probes to on the metal particles, positioning of the fluorophore near the metallic surfaces and the detection of *Bacillus anthracis*. Applicants vigorously disagree because Lockhart

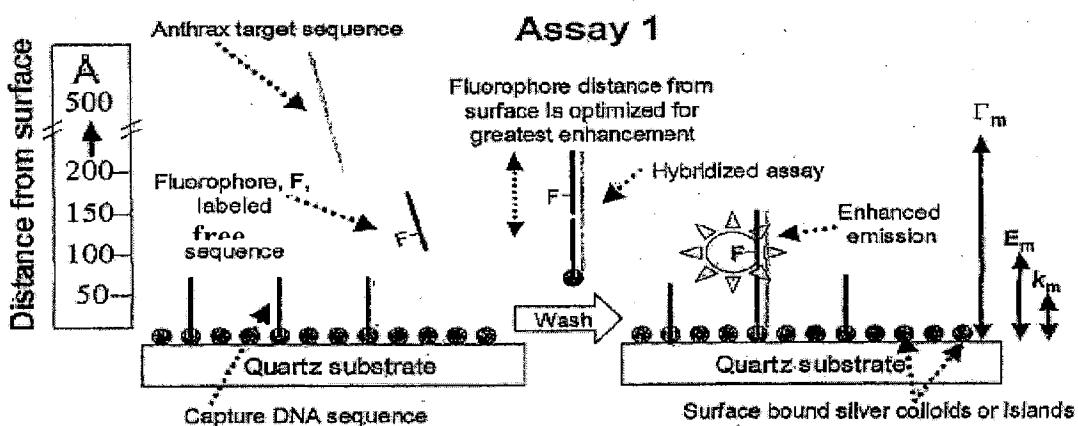
alone or in combination with the other references does not teach the components of the presently claimed invention.

Applicants' claimed invention as recited in claim 1 include:

- (1) a substrate with immobilized metal particles, wherein the metal particles include a covalently bonded captured nucleotide sequence probe complementary to a first known sequence of a nucleotide sequence of the *B. anthracis*, and
- (2) a free nucleotide sequence probe, wherein the free nucleotide sequence probe has been fabricated to a second known sequence of the *B. anthracis* and having an affinity for said nucleotide sequence of *B. anthracis*, wherein a fluorophore is attached to the free nucleotide sequence,
- (3) both of these two probes are necessary to determine if a test sample includes the nucleotide sequence of *B. anthracis*.



It should be very clear that the nucleotide sequence of *B. anthracis* is known so the two probes include sequences that are complementary to two different regions of the known anthrax sequence and are prepared to attach to different regions of the *B. anthracis* sequence. Clearly if there is no *B. anthracis* sequence in the sample then the captured nucleotide sequence probe will remain unbound, and thus, the free probe will not bind to anything and in turn—no signal. In the alternative, if there is *B. anthracis* sequence in the sample then it will attach to the captured nucleotide sequence probe and then the free nucleotide sequence probe will bind to the second site that is complementary to the free nucleotide sequence probe and a signal produced. Thus applicants' system includes the use of two separate and distinct probe sequences (a captured and free nucleotide sequence) that are complementary to known different sections of the target nucleotide sequence. The free nucleotide probe sequence includes a fluorophore positioned a distance from the metal surface. This is shown below by applicant's Figure 1.



Importantly, the use of the two probes that have affinity for different nucleotide sequences in the target sequence, under high stringency conditions, is advantageous because it allows for increased sensitivity. Thus, when both probes are bound to the target sequence there is very little doubt regarding the identity of the bound sequence. In fact using the two probes provides for additional verification that the target sequence is indeed anthrax.

The Office makes reference to several sections of the Lockhart reference and specifically page 71 and Figures 12 and 13. However, the text considered by the Office does not in any way teach all the required components.

Lockhart teaches a method for identifying differences in nucleic acid abundances. The methods and systems include an array containing a large number (greater than a 1000) of arbitrarily selected different oligonucleotide probes where the sequence of the probe is known and the exact location in the assay is known. Thus, there is a multiplicity of different capture probes. This large number of probes is essential so that differences in the hybridization patterns can be used to determine the differences in the expression of various genes.

It is very clear that Lockhart teaches the use of an array of different probes and states on page 51 that the probes can be random, arbitrary haphazard, composition biased or include all possible oligonucleotides of a particular length. Further on page 53, the reference states that the invention can include 1,000,000 different probes, to provide every probe of a characteristic length that binds to a particular nucleic acid sequence. On page 71, there is a discussion that the probes can include a constant region but if they do they MUST also include a variable region which again provides for the required randomness. Thus, none of the probes used in the Lockhart system provide for a probe that includes an entire sequence complementary to a single known sequence of the target pathogen as in the present invention. Clearly, with all the possible lengths of the probes, there is no chance of continuity or the possible placement of a fluorophore at a specific distance from a metallic particle. The present invention demands continuity, that being, a single probe that has affinity for a single sequence area on the target pathogen which will allow the second free probe to attach at the optimal position.

Further, regarding labeling of the target nucleic acid in Lockhart, the labeled probes must include a ligatable oligonucleotide and ligase. For determination of the labeled sequence, there must be a ligase involved so that the probe and labeled nucleotide sequence can be ligated together as shown in Figure 12. Thus, the two sequences have to be sufficiently close to allow such ligation and it is very apparent the label must be at the end of 5' end of the ligatable oligonucleotide. Clearly, there is nothing in this reference that discloses or even recognizes the importance of placement of the label for interaction with metallic particles on the substrate.

According to the Office, Lakowicz teaches a method for increasing the fluorescence of a fluorophore by using metal particles. However, the Lakowicz reference does not provide any indication of placement of a fluorophore on a nucleotide sequence that provides any guidance to go

in the direction of applicants' claimed invention. Instead there is a discussion of intrinsic DNA fluorescence molecules that do not include an external fluorophore. The only discussion relating to the addition of an extrinsic fluorophore to the DNA is negative as discussed on page 101 at the bottom of column 1, wherein the text expressly states that using extrinsic fluorophores introduces complications including the limiting factor of having to label the DNA.

Increased intrinsic emission from DNA may provide new approaches to DNA analysis.

One possibility is single-strand DNA sequencing.<sup>8</sup> The goal is to use exonuclease cleavage of terminal DNA bases that are identified after their sequential release.

A limiting factor in this approach will be the reactive yield of labeling each nucleotide with an extrinsic fluorophore after its release.

Instead, an appropriately designed flow chamber employing metallic particles could enhance the base emission by a combination of the lightning-rod effect, increased radiative rate, decreased lifetime and increased photostability, all contributing to more photons per released base (Figure 6).

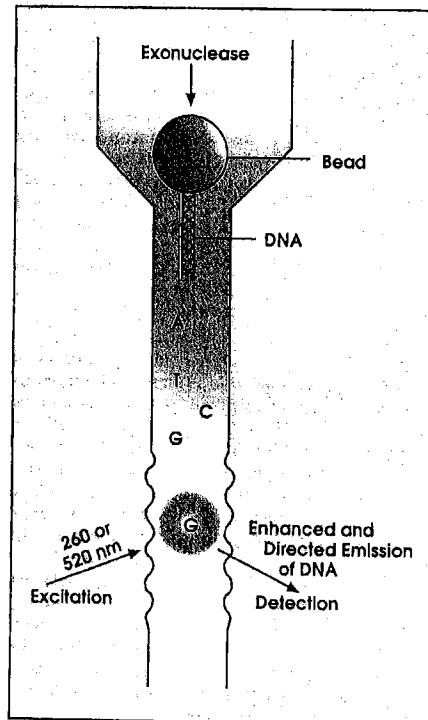


Figure 6. To simplify single-strand DNA sequencing, metal particles could enhance the intensity and provide directionality for emissions of unlabeled nucleotides.

Instead the reference uses the intrinsic method and as discussed in Figure 6, recreated above, only unlabeled nucleotides are used. Thus, it is evident that the Lakowicz reference does not provide any information regarding labeling a DNA nucleotide probe with a fluorophore that is positioned a specific distance from metallic particles.

Initially, it should be noted that Lockhart provides for labels attached to the 5' terminus end of a nucleotide sequence but Lakowicz teaches that additional labeled fluorophores are a problem and instead uses DNA as the intrinsic fluorophore. There is nothing in Lockhart that teaches the use of intrinsic fluorophore, that being just the DNA having intrinsic fluorescence. One skilled in the art would never consider using the teaching of Lakowicz in combination with the

Lockhart system because it is very evident that the Lockhart system would no longer operate as intended or it could change the mode of operations. Clearly, the label is essential to determine if the ligase provided the necessary ligation and if the label is not there, then the expected hybridization did not occur.

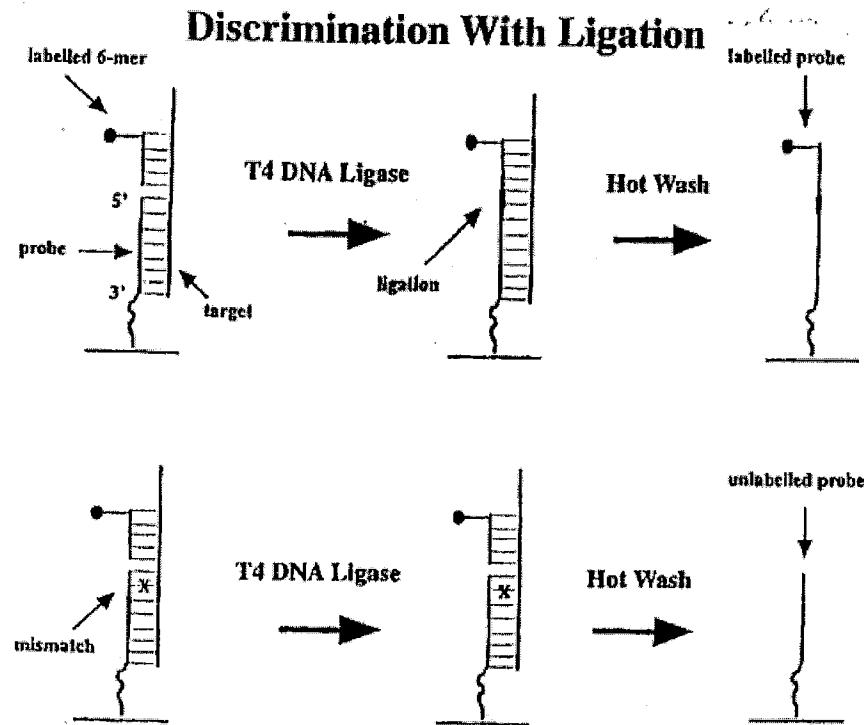


Fig. 12

The MPEP § 2143.01 V – VI states that:

“If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. ... [and] If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious.”

Further, according to the court in *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984), if proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification and the Office has not established a *prima facie* case of obviousness.

The Office has cited several other references in an attempt to establish a *prima facie* case of obviousness, however the addition of Cao or Qi does not rectify the shortcomings of the Lockhart and Lakowicz combination. If none of the prior art teaches or suggests all the claimed components then the prior art does not defeat the patentability of claims 1, 4-10, 12-16 and 18-27. Applicants request the withdrawal of this rejection under section 103.

2. Claims 1, 4-10, 12-16 and 18-27 were rejected under 35 U.S.C. 103(a) as being unpatentable over Cao as evidenced by Malicka and Lukomska in view of Lakowicz 1 and 2. Once again applicants insist that the proposed combination does not defeat the patentability of the presently claimed invention.

Initially, it should be noted that applicants' application has a priority date of November 26, 2002 and to establish a *prima facie* case of obviousness, the cited prior art has to be available to the skilled artisan at the time of filing. Thus any prior art cited has to be available before the November 26, 2002. Clearly, the Malicka reference with a date of 2003 (Biopolymers (2003) 72(2) 96-104) and the Lukomska reference with a date of 2005 (Biopolymers and Biophysical Research Communication (2005) 328: 78-84) do not meet this requirement. As such, applicants are not even considering them in this response because neither reference is competent prior art.

According to the Office, Cao is a primary reference and when combined with a multiplicity of other references teaches the presently claimed invention. This Cao reference published on August 30, 2002 and applicants have an effective filing date of November 26, 2002 (provisional application) which is clearly within a one year period of the Cao publication. Thus, it is evident that applicants have the right to swear behind this reference. As such, applicants submit herewith is a Declaration executed by the inventors showing conception, diligence and reduction to practice, with evidence found in Appendix A.

The Declaration attests to facts showing conception of the claimed invention prior to the publication date of Cao reference and diligence immediately before the publication date, with effective reduction to practice after the publication date with the filing of a Disclosure Document at the University attesting to such reduction and subsequent filing of the provisional application on November 26, 2002 in a timely fashion.

Reference	Effective Date
Cao	August 30, 2002

The Declaration includes appended Exhibits 1 and 2.

Exhibit 1 is a copy of power point slide having a date of creation and modification, that have been blackened out, prior to the August 30, 2002 publication date of the Cao reference. Exhibit 1 clearly shows the use of the claimed assays of the present invention wherein the two nucleotide probes are used to bind with a target sequence of anthrax.

Exhibit 2 is a copy of the Disclosure and Record of Invention submitted by the inventors after the Publication date of August 30, 2002 and before the filing of the provisional application on November 26, 2002.

Exhibits 1 and 2 in addition to the enclosed Declaration provide evidence of conception before the effective date of August 30, 2002 and actual reduction to practice subsequent to the effective date. As such, applicants request that the Cao reference be removed as a competent reference.

The Office has also cited the two Lakowicz references, that being, both Lakowicz 1 and Lakowicz 2. Notably neither Lakowicz 1 nor Lakowicz 2 is sufficient to establish a *prima facie* case of obviousness because the combination does .

In light of the foregoing discussion and the fact that all of claimed limitations are not disclose it is clear that the Office has not met its burden of establishing a *prima facie* case of obviousness.

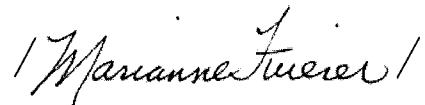
#### **Fees Payable**

Applicants petition for a one month extension and the fee for such extension is being paid by electronic transfer. If any additional fee is found due for entry of this amendment, the Commissioner is authorized to charge such fee to Deposit Account No. 13-4365 of Moore & Van Allen.

**Conclusion**

Applicants have satisfied the requirements for patentability. All pending claims are free of the art and fully comply with the requirements of 35 U.S.C. §112. It therefore is requested that Examiner Bertagna reconsider the patentability of the pending claims in light of the distinguishing remarks herein, and withdraw all rejections, thereby placing the application in condition for allowance. If any issues remain outstanding incident to the allowance of the application, Examiner Bertagna is requested to contact the undersigned attorney at (919) 286-8089.

Respectfully submitted,



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## **APPENDIX A**